PROMOTING A CLEAR, BALANCED UNDERSTANDING OF BIOTECHNOLOGY

PUBLIC UNDERSTANDING OF BIOTECHNOLOGY

GENETICALLY MODIFIED ORGANISMS AND WINE

The South African wine industry

The South African wine industry is composed of eight wine regions which produced a total of 1044 million litres of wine in 2007. Fifty seven percent of these wines were consumed locally and 87.1 million litres (28% of total exported volume) were exported to the UK, South Africa's single largest export market. The total producer income for 2007 was R 2.9 billion. South Africa is currently ranked the ninth largest wine producer in the world, yielding 3% of the total annual production (OIV Statistics, 2005). The four largest wine producers are, in order of world ranking, Italy, France, Spain and the USA (SAWIS, 2007). Several white and red wine grape cultivars are grown on approximately 102 000 hectares in South Africa, each offering a unique product depending on the cultivar, specific region and wine style.

The science of wine making

Although wine making is seen by many as an art, it is based on specialised physical and biological sciences, specifically chemistry, biochemistry and microbiology. Three different living organisms, i.e. grapes, yeast and bacteria are used in the process of making wine. The wine industry is therefore a biotechnology-based industry.

The process begins with red or white wine **grapes** (*Vitis vinifera*) that are specifically selected for the preparation of wine because of their characteristic flavours and aromas. Grapes are harvested, destemmed and crushed to form wine must – a mixture of grape juice and berry skins (reds). To initiate the process of alcoholic fermentation a selected wine **yeast** strain from the species *Saccharomyces cerevisiae* with specific characteristics, is added to the must. During the fermentation process, the yeast cells convert the grape sugars into alcohol and also produce several other chemical compounds that contribute to the final characteristics of the wine. To decrease the acid content and enhance the aroma profile of red and some white wines, **lactic acid bacteria**, usually *Oenococcus oeni*, is added after the initial fermentation process to convert malic acid into lactic acid - a process called malolactic fermentation. The wine is now ready for filtration, bottling or aging (Van Rensburg, 2001).

All the processes involved in the preparation of wine have been thoroughly researched over many decades. With the advancement in knowledge and a better understanding of these processes, wine-makers can now consistently produce wines of higher quality. All three of the wine organisms mentioned above have been subjected to improvement programmes that include breeding, mutagenesis and more recently direct genetic improvement in different research groups around the world.

Improvement of grapevine

Grapevine breeding programmes have always been aimed at improving agronomic traits, especially the health of the plants, and enhancing the winemaking properties of the fruit.
The effectiveness of these programmes has improved through the years due to the development of new analytical techniques and a better understanding of grapevine genetics. Standard grapevine breeding techniques have been successful, but are extremely expensive, complex and time consuming. Vines only bear sufficient fruit for testing and selection of berry characteristics after a youth phase of three or more years after planting and a detailed assessment of features such as wine making potential can take up to 25 years. A further problem associated with wine grape breeding is the necessity to establish new varietal names, which in the wine industry refers directly to the particular character of the final product: wine. The new and improved cultivar thus loses the marketing advantage of

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the name of its parents. In contrast, however, genetic engineering can be used to introduce selected genes without disrupting the varietal characteristics, and consequently the name of the cultivar may not need to be changed (Jackson, 2000). For example, introduced disease resistance should have no direct impact on the characteristics of the fruit.

A major breakthrough for grapevine characterisation and improvement was the sequencing of the entire genome by researchers in France and Italy in 2006 (Jailon *et al*., 2007; Velasco et al., 2007). This information can aid researchers in the areas of breeding and genetic engineering in identifying and using genes associated with specific characteristics of the plant. Genetic engineering has enormous application potential in the improvement of grapevine, based on the market demand for cost-effective, sustainable and environmentally friendly production of healthy, top quality grapes and wine (Vivier and Pretorius, 2002).

Specific targets for grapevine genetic engineering

Several countries worldwide, including South Africa, have already developed transgenic (genetically modified or GM) grapevines to address problems specific to their environment and needs. The major target for direct genetic improvement of grapevine is the production of plants that are resistant to pests and diseases. Grapevine health is threatened by several fungal, bacterial and viral diseases and substantial advances in the development of resistant genetically modified (GM) grapevine lines have been made in Chile, France, Germany, South Africa and the USA (Vigne *et al*., 2004; gmoinfo.jrc.it/gmp_browse.aspx). Several greenhouse and field trials are currently underway, the latter being essential for the effective evaluation of the plants because natural field conditions cannot be reproduced in greenhouses. Increased resistance to pathogens and other pests could result in the reduced use of agrochemicals and fungicides and will have a positive impact on the health of farm workers, conservation of the environment and production costs.

Other targets of international GM grapevine programmes include improved fruit quality and environmental stress resistance. Basic quality factors such as colour and sugar development are improved by different mechanisms in GM grapevine plants in Australia (www.ogtr.gov.au/ir/dir031), Italy (Costantini *et al*., 2007) and South Africa. The development of grapevine plants with the ability to adapt to adverse environmental conditions, including drought, salt and high light intensity stress is also receiving attention in Argentina and South Africa (Vivier and Pretorius, 2002; gmoinfo.jrc.it/gmp_browse.aspx).

What is the situation in South Africa?

The South African grapevine biotechnology programme is funded by the wine and table grape industries. It focuses on the development of fungal and viral resistant vines, and the metabolic engineering of grapevine towards enhanced environmental stress resistance and improved grape berry quality factors such as colour and aroma (www.sun.ac.za\wine_biotechnology). Several transgenic grapevine lines are currently being evaluated in greenhouse trials. In 2006 researchers at the Institute for Wine Biotechnology (IWBT) at Stellenbosch University (SU) applied for a permit to perform the first GM grapevine field trials in South Africa. They proposed to graft transgenic grapevine plants on non-transgenic rootstocks. The objectives of the trial were to evaluate the morphology, growth and fruit quality of the transgenic plants under field conditions (SU press release, 2006). The application was evaluated by the Advisory Committee (GMO Act) and a list of questions regarding the trial was referred back to the applicant. The response of the applicant is still outstanding and the final decision of the Executive Council (EC) is pending (EC meeting minutes, September 2007).

Improvement of wine yeast

In modern wineries, reliable fermentations are essential to obtain consistent wine flavour and predictable quality and therefore specially selected starter culture strains of *Saccharomyces cerevisiae* are used. In addition to the primary function of these wine yeast strains, namely fermentation, they can also have a range of specialised properties that can add value to the final product. This demand for wine yeast strains that are optimised for specific tasks set by winemakers has led to dedicated yeast breeding and genetic engineering (Pretorius and Bauer, 2002). Sequence data of the complete *Saccharomyces cerevisiae* genome and recent advances in wine sciences now enables researchers to comprehensively assess the modifications that are engineered into the yeast cells. In a study where genetically modified yeast strains were compared to non-GM yeast strains, it was found that the differences were no more than the normal variation that is usually observed between yeast strains bred from different parents (Bauer *et al*., 2004).

Specific targets for yeast genetic engineering

Five major targets for the direct genetic improvement of wine yeast strains have been identified, all relating to the improvement of the winemaking process and wine quality by enhancing the following characteristics:
1) efficiency of the fermentation process,
2) processing of wine, 3) wholesomeness,

4) sensorial quality and 5) control of microbial spoilage (Pretorius and Bauer, 2002).

Genetically engineered wine yeast strains are being developed in most wine-producing countries including Canada, France, Germany, Italy, Portugal, South Africa, Spain, Sweden and the USA. Several studies have already shown the feasibility of improving wine yeast through genetic engineering for traits such as the secretion of specific enzymes that improve wine clarification and juice yield, the production of aroma compounds and the decrease of ethanol production (Bauer et al., 2007). However, only two genetically modified yeast strains have to date been released for commercial use; a malolactic yeast, ML01 (Husnik et al., 2007), and a yeast that produces lower amounts of the carcinogen, ethyl-carbamate (Coulon et al., 2006). Both of these yeast strains have been authorised for use in the North American wine industry (Bauer *et al*., 2007).

What is the situation in South Africa?

The Institute for Wine Biotechnology at Stellenbosch University is a world leader in the field of wine yeast strain development (www.sun.ac.za\wine_biotechnology). Several novel yeast strains (GM and non-GM) have already been developed (Bauer *et al*., 2007) but no GM wine yeast is currently used for wine making in South Africa. The research is funded in part by the South African wine industry.

The GM malolactic yeast, ML01 that performs both alcoholic and malolactic fermentations simultaneously, thereby minimising the use of lactic acid bacteria that produce off flavours and allergens in wine, has been developed by researchers in Canada. ML01 has received "Generally Regarded as Safe" (GRAS) status from the FDA, the United States regulator (www.landfood.ubc.ca/wine/vanvuuren/vanvuuren). In 2006 an application was submitted to the Registrar: GMO Act for the commercialisation of ML01 in South Africa. No health or environmental concerns were raised but the application was, however, denied by the EC due to socio-economical concerns. The South African Wine Industry, as represented by the SA Wine Industry Council, did not support the release of the yeast strain in the industry. The concerns included perceived consumer resistance to GMOs and the perception that South Africa's major export markets in the UK and Europe are opposed to using GM organisms in wine preparation. The wine industry was concerned that the image of South African wines would be irreparably harmed (EC meeting minutes, July 2006).

Improvement of lactic acid bacteria

Lactic acid bacteria (LAB) play a very important role in winemaking because of the deacidification and characteristic aroma profile it creates in the wine. LAB, especially *Oenococcus oeni*, is used for malolactic fermentation of wine. LAB also produces antimicrobial agents that can inhibit the growth of spoilage LAB and thus might decrease the levels of sulphur dioxide used in wine. The major target of the LAB strain development programme is to select for strains that are better adapted as starter cultures for malolactic fermentation (Bauer *et al*., 2007). The complete genome sequence of *Oenococcus oeni* was published in 2005 (Mills *et al*., 2005) and direct genetic improvement protocols for this bacterium are also currently being developed. Countries involved in the improvement of lactic acid bacteria strains for wine making includes Australia, France, Germany, Italy, South Africa and the USA.

What is the situation in South Africa?

No genetically modified LAB strains have been developed in South Africa to date. Research efforts at the IWBT are focused on specific enzymes that are involved in the production of wine aroma compounds and bacteriocins that can be used as alternative to chemical preservatives. LAB is also regarded as a spoilage microorganism and researchers are investigating the production of bitterness, biogenic amines and compounds causing off-flavours in wine. The research findings will be useful in the identification of target genes for the future direct genetic improvement of LAB strains (www.sun.ac.za\wine_biotechnology).

Is there international regulation/ legislation for the use of GM technology in wine?

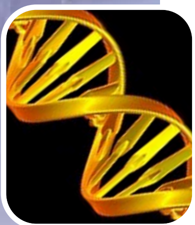
The International Organisation of Wine and Vine (OIV) is an inter-governmental organisation that aims to contribute to the international harmonisation of existing practices and standards concerning vines, wine, wine-based beverages, table grapes, raisins and other vine-based products. The OIV prepares new international standards in order to improve the conditions for producing and marketing vine and wine products, and helps ensure that the interests of consumers are taken into account. Membership to the OIV is voluntary and its recommendations are viewed as guidelines only. The organisation does not have the authority to instigate laws regarding production practices in the individual member countries.

The OIV is currently still in the process of developing a GMO policy which will set the guidelines and standards for the use of GMOs in wine production (OIV Resolution VITI 1/2006). It does currently support research activities involving GM organisms but not the commercial use thereof in wine making. South Africa is a member of the OIV and will adhere to these guidelines. The USA and Canada, where the GM yeasts have been approved for commercial use, are not members of the OIV (www.oiv.int\uk).

What is the opinion of the South African Wine industry on the development of GM wine organisms?

The South African wine industry has long since appreciated the potential of biotechnology and the development of genetically modified wine organisms, and has been funding research in this area since 1991. They also realise that currently the biggest risk of this technology is the non-acceptance of GM products, vines, yeasts and to a smaller extent enzymes and bacteria in the production of alcoholic beverages (Groenewald, 2006). The SA Wine Industry Council stated in a press release in 2006 that they will not use GM organisms in the production of wine in South Africa until this practice is internationally acceptable (www.sawb.co.za/docs/gmo.pdf). The industry will, however, continue to support and fund GM wine research through its Winetech Biotechnology Programme (www.winetech.co.za) in line with its research policy in order to remain at the cutting edge of the technology. Increased global competition, shifts in consumer demands and expectations, in particular regarding wine quality, as well as a need to implement more environmentally friendly production practices are the driving forces of the industry to support this technology (Pretorius and Bauer, 2002).




Public concerns regarding GM grapevine and wines

The application of GM wine technologies have raised the same general safety concerns regarding genetically modified organisms (GMOs) and include the long term effect on human health and the environment. The South African GMO regulatory system requires that thorough risk assessments of GM products in terms of human health, environmental and socio-economical risks are performed before general release. However, because wine can be described as a life style product consumers are also concerned that the unique character of the drink will be lost. According to Professor Sakkie Pretorius, a world-renowned wine biotechnologist, the benefits of direct genetic improvement of wine organisms will be realised only if "the application is judicious, systematic and achieved with high regard for the unique nature of the product. The first GM wine products should unequivocally demonstrate organoleptic (colour and aroma), hygienic and economic advantages for the wine producer and the consumer. Furthermore, wine's most enthralling and fascinating aspect, its diversity of style, should never be threatened by the use of tailored wine yeasts. In fact, gene technology should rather be harnessed to expand the diversity of high-quality wines" (Pretorius and Bauer, 2002).

References:

1. Bauer, FF; Naes, T; Esbensen, K; Young PR, Du Toit, M and Vivier, MA. 2007. Functional wine-omics. Blair, R.J.;Pretorius, I.S. (eds). *Proceedings of the Thirteenth Australian Wine Industry Technical Conference*, 29 July to 2 August 2007, Adelaide, South Australia. pp 178-183.
2. Bauer, FF; Dequin, S; Pretorius, IS; Shoeman, H; Wolfaardt, G; Schroeder MB and Grossmann, MK. 2004. The assessment of the environmental impact of genetically modified wine yeast strains. *Bulletin de l'OIV* 77: 515-528.
3. Costantini, E; Landi, L; Silvestroni, O; Pandolfini, T; Spena, A and Mezzetti B. 2007. Auxin synthesis-encoding transgenes enhances grape fecundity. *Plant Physiology* 143:1689 – 1694.
4. Coulon, J; Husnik JI; Inglis, DL; Van der Merwe, GK; Lonvaud, A; Erasmus DJ and Van Vuuren, HJJ. 2006. Metabolic engineering of *Saccharomyces cerevisiae* to minimize the production of ethyl carbamate in wine. *Am. J. Enol. Vitic.* 57:113-24.
5. Deliberate releases and placing on the EU market of Genetically Modified Organisms (GMOs). Biotechnology and GMO information website. http://gmoinfo.jrc.it/gmp_browse.aspx
6. Executive Council (GMO Act) meeting minutes. July and September 2007. www.nda.agric.za.
7. Groenewald, S. 2006. The second Winetech Biotechnology Information Day. *Wynboer* 197: 87-88.
8. Husnik, JI;, Delaquis, PJ; Cliff, MA and van Vuuren, HJJ. 2007. Functional analyses of the malolactic wine yeast ML01. Am. J. Enol. Vitic. 58:42-52.
9. Information system for biotechnology. 2008. www.isb.vt.edu
10. Institute for Wine Biotechnology. www.sun.ac.za\wine_biotechnology
11. Jackson, RS. 2000. Grape species and varieties. In *Wine Science: Principles, Practice, Perception*, Academic Press, ISBN:012379062X, pp 27 – 34.
12. Jaillon, O, et al. French-Italian Public Consortium for Grapevine Genome Characterization. 2007 The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* 449:463-467.
13. Mills, DA; Rawsthorne, H; Parker T; Tamir, D; Makarove, K. 2005. Genomic analysis of Oenococcus oeni PSU-1 and it relevance to winemaking. *FEMS Microbiol. Rev.* 29: 465-475.
14. OIV (Organisation Internationale de la Vigne et du Vin) Resolution VITI 1/2006 on Vine genome and genetically modified varieties. www.oiv.int/uk/accueil/index.php.
15. OIV Statistics, 2005, www.oiv.int/uk/accueil/index.php.
16. Pretorius, IS and Bauer, FF. 2002. Meeting the consumer challenge through genetically customized wine-yeast strains. *Trends in Biotechnology* 20(10):426 – 432.
17. Pretorius, IS and Høj, PB. 2005. Grape and Wine Biotechnology: Challenges, opportunities and potential benefits. *Australian Journal of Grape and Wine Research* 11:83-108.
18. South African Wine Industry Council Press release, 17 October 2006. www.sawb.co.za/docs/gmo.pdf.
19. South African Wine Industry Statistics Nr 31. 2007. *SA Wine Industry Information Services* (SAWIS; www.sawis.co.za).
20. Stellenbosch University (SU) press release, 7 September 2006, *Cape Argus* p13
21. Van Rensburg, P. 2001. Wynbereiding. *Oenology course material*, Stellenbosch University.
22. Velasco, R. *et al*. Agricultural Institute of San Michele all'Adige. 2007 A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. *PLoS ONE 2*(12): e1326. doi:10.1371/journal.pone.0001326.
23. Vigne, E; Komar, V and Fuchs, M. 2004. Field safety assessment of recombination in transgenic grapevines expressing the coat protein of Grapevine fanleaf virus. *Transgenic Research* 13: 165 – 179.
24. Vivier, MA and Pretorius, IS. 2002. Genetically tailored grapevines for the wine industry. *Trends in Biotechnology* 20: 472-478.
25. Wine Research centre, University of British Columbia, Prof HJJ van Vuuren. 2008. www.landfood.ubc.ca/wine/vanvuuren/vanvuuren
26. Wine Industry Network of Expertise and technology. www.wintech.co.za

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